PATENT

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In re application of:
Gerard T. Hardiman *et al.*

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Title:
HUMAN RECEPTOR PROTEINS; RELATED REAGENTS AND METHODS

DECLARATION UNDER 37 C.F.R. § 1.132

I, Terrill McClanahan, hereby declare as follows:

1. I received a PhD degree in Biological Chemistry from the University of California, Los Angeles, California, in 1986. I have extensive experience in the area of molecular biology, as evidenced by my attached Curriculum Vitae.

2. I am currently employed by DNAX Research Institute (DNAX), a subsidiary of Schering-Plough Corporation, as a Senior Staff Scientist.

3. As a DNAX employee, I have performed experiments relating to DNAX Toll-Like Receptor 6 (DTLR6), which is known in the public nomenclature as Toll-Like Receptor 7 (TLR7). I am submitting the below data to demonstrate that TLR7 expression is elevated in immunological disorders, such as psoriasis and atopic dermatitis.

4. The purpose of these experiments was to determine TLR7 expression levels in skin samples from patients afflicted with psoriasis and atopic dermatitis. All tissues were screened in-house by consulting pathologists. Staging diagnoses were confirmed. Real-time quantitative PCR values were normalized to ubiquitin. Kruskal-Wallis statistical analysis was performed on log transformed data (median method).

5. The human inflammatory skin disease panel included normal skin, and non-lesional and lesional skin from both psoriasis and atopic dermatitis patients. The first panel included 35 normal skin samples, 24 non-lesional psoriasis skin samples, 25 lesional psoriasis skin samples, 30 non-lesional atopic dermatitis skin samples and 30 lesional atopic dermatitis skin samples. Two 4mm punch biopsies were taken from each patient. Total RNA was isolated and real-time PCR was performed using standard methods.

6. All non-lesional and lesional patient samples were ranked by severity using either the PASI (Psoriasis Area and Severity Index) score or EASI (Eczema Area and Severity Index)

score. For psoriasis patients, the PASI scores were in the range of 9 to 20.75. For atopic dermatitis patients, the EASI scores were in the range of 1.85 to 35.95. These scores reflected the extent and severity of disease over the patient's body. Real time quantitative PCR values were normalized to ubiqutin. Kruskal-Wallis statistical analysis was performed on log transformed data (median method).

7. The normal skin tissue samples (n = 35) had an average TLR7 expression value of 3.63, while the psoriasis non-lesional, psoriasis lesional, atopic dermatitis non-lesional, and atopic dermatitis lesional skin tissue samples had average TLR7 expression values of 5.85 (a 1.6 fold increase), 10.12 (a 2.8 fold increase), 5.41 (a 1.4 fold increase) and 5.42 (a 1.4 fold increase), respectively. Kruskal-Wallis median analysis on log-transformed data showed significant elevation in both non-lesional and lesional psoriasis, with P values of less than 0.01 and less than 0.001, respectively. TLR7 expression was elevated in the non-lesional atopic dermatitis samples, with a P value of less than 0.05, and not statistically elevated in the lesional atopic dermatitis samples.

8. The above experimental results demonstrate that TLR7 expression is elevated in psoriasis and atopic dermatitis skin samples compared to normal skin samples and points to the usefulness of TLR7 nucleotide sequences in the diagnosis of psoriasis and atopic dermatitis.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application and any patent issued thereon.

Terrill McClanahan

1-26-04
Date

Curriculum Vitae

Terrill Ann Klooster McClanahan

Birthdate: April 26, 1960

Birthplace: Escondido, California

Education:

Pacific Union College	Angwin, California	
B. S. Chemistry	June 1981	
University of California	Los Angeles, California	
Ph. D. Biological Chemistry	December 1986	

Honors: Vernon Winn Chemistry Award
Pacific Union College, 1981

Research Support:

NIH/NRSA Cell and Molecular Biology pre-doctoral training grant GM 07185-08 9-1-81 to 8-31-84 (3 yr. award)

NIH/NRSA Genetics post-doctoral research fellowship award GM 11877-01 9-1-87 to 5-1-89 (3 yr. award)

Research and/or Professional Experience:

9/81-11/86	Graduate Student DNA-damage responsive genes in *S. cerevisiae* Laboratory of Dr. Kevin McEntee UCLA Medical School, Dept. of Biol. Chemistry Los Angeles, California
1/87-5/89	Postdoctoral Fellow Growth-regulated expression of the human HSP70 gene Laboratory of Dr. Richard Morimoto Department of Molecular and Cell Biology Northwestern University Evanston, Illinois
5/89-2/92	Postdoctoral Fellow Gene Expression of Hematopoietic Development

Laboratory of Dr. Frank Lee
DNAX Research Institute
Palo Alto, California

2/92-5/94 Senior Research Associate
Strategies for Cloning Novel Genes Involved in Hematopoietic Lineage Commitment
Laboratory of Dr. Frank Lee
DNAX Research Institute
Palo Alto, California

5/94-7/96 Senior Research Associate
Group Leader, Novel Cloning Group
Supervisor: Dr. Gerard Zurawski
DNAX Research Institute
Palo Alto, California

7/96-9/00 Staff Scientist
Group Leader, Novel Cloning Group
Supervisor: Dr. Gerard Zurawski
DNAX Research Institute
Palo Alto, California

9/00-12/00 Senior Staff Scientist
Group Leader, Novel Cloning Group
Supervisor: Dr. Gerard Zurawski
DNAX Research Institute
Palo Alto, California

1/01-present Senior Staff Scientist
Group Leader, Molecular Pathology Team
Supervisor: Dr. Robert Fick
DNAX Research Institute
Palo Alto, California

Publications

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